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厦门大学

硕 士 学 位 论 文

microRNA 在皮肤和角膜上皮干细胞的差异性表达及其功能的初步研究

Differential expression of microRNAs between LESCs and SESCs and pilot study of their functions

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论文提交日期： 2014 年 4 月

论文答辩日期： 2014 年 5 月

2014 年 4 月

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英文缩略语对照表

Abbreviation	Full Name
BSA	albuminbovine
cDNA	complementary DNA
DMSO	dimethyl sulfoxide
EDTA	ethylene diamine tetraacetic acid
EGF	epidermal growth factor
FAM	carboxyfluorescein
FBS	fetal bovine serum
HCE	human corneal epithelial cells
HEPES	N-(2-hydroxyethyl)piperazine-N-2-ethancsulfonic acid
ITS	insulin-transferrin-selenium
K1/3/5/10/12/14/15/19	cytokeratin 1 /3/5/10/12/14/15/19
KSFM	keratinocyte serum-free medium
LESCs	limbal epithelial stem cells
MMC	mitomycin C
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
microRNA	micro ribonucleic acid
mRNA	messsage ribonucleic acid
NC	negative control
Pax6	paired box gene 6
PBS	phosphate buffered saline
PFA	paraformaldehyde
PVDF	immobilon-P transfer membrane
p38	p38 mitogen-activated protein kinases
p63	tumor protein 63
Realtime PCR	realtime polymerase chain reaction

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RT-PCR	reverse transcription polymerase chain reaction
SDS	sodium dodecyl sulfate
SESCs	skin epithelial stem cells
SHEM	supplemental hormonal epithelial media
SSC	sodium chloride-sodium citrate
TEMED	tetramethylethylenediamine
UTR	untranslated region

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摘 要

目的:通过基因芯片技术找出角膜上皮干细胞和皮肤上皮干细胞差异性表达的 microRNAs 并对其在增殖和分化方面的功能及作用机制进行初步的研究和探讨。

方法:

1. 运用克隆培养方法分别培养三组人角膜和皮肤上皮干细胞，进行 microRNA 基因芯片分析，找出有显著差异性表达的 microRNA($p \leq 0.05$ 差异倍数≥ 1.5)。

2. 对基因芯片分析出的差异性表达的 microRNA 进行荧光实时定量 PCR，筛选出表达模式与芯片结果一致并且差异倍数较大的 microRNA。

3. 运用荧光实时定量 PCR，研究筛选出来的有显著差异的 microRNA 在角膜和皮肤上皮干细胞分化过程中的变化。

4. 利用瞬时转染的方法将在角膜缘干细胞中显著表达上调的 miR-145 mimics 转染进入皮肤上皮干细胞，用相差显微镜观察细胞形态变化，MTT 检测细胞增殖能力的变化。

5. 用 TargetScan 及 miRanda 预测 miR-145 与增殖相关的靶基因，荧光实时定量 PCR 和蛋白免疫印迹（Western blot）研究证实过表达 miR-145 后其靶基因及靶基因下游基因的变化。

结果:

1. microRNA 基因芯片分析共找出了 27 个在角膜上皮干细胞和皮肤上皮干细胞中差异性表达的 microRNA；其中在角膜上皮干细胞中表达较高的有 17 个，为 miR-30a，miR-1297，miR-4301，miR-4262，miR-199aa-3p，miR-190，miR-302f，miR-338-5p，miR-484，miR-21，miR-376b，miR-26a，miR-140-3p，miR-18b，miR-145，miR-652，miR-324-5p；在皮肤上皮干细胞中表达较高的 10 个，为 miR-23a*，miR-943，miR-1236，miR-193b*，miR-382，miR-542-3p，miR-205*，miR-224*,miR-378，miRPlus-1382*。

2. 实时荧光定量 PCR 验证出与基因芯片结果表达模式一致且差异倍数较大的 microRNA 有 8 个（miR-4262，miR-199aa-3p，miR-190，miR-302f， miR-21，miR-376b，miR-140-3p，miR-145），均为在角膜缘上皮干细胞中显著上调表达的 microRNA。

3. 对角膜和皮肤干细胞分化过程筛选出的 8 个差异性表达的 microRNA 变化的

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分析中发现，其中 6 个在角膜和皮肤上皮干细胞分化过程中表达均显著降低，而仅 miR-302f 和 miR-4262 在角膜上皮干细胞分化过程中显著升高，而在皮肤上皮干细胞分化过程中显著降低。

4. 选取 miR-145 进行初步的功能研究，结果表明 miR-145 可以抑制皮肤上皮干细胞的增殖能力。
5. miR-145 抑制皮肤上皮干细胞增殖能力的功能是通过下调 p38 基因的表达从而抑制 p38 MAPK 信号通路来实现的，同时信号通路下游基因 p63 的表达也降低。

结论：

1. 差异性表达的 microRNA 在维持角膜和皮肤上皮干细胞各自的特性中可能起到关键作用。
2. 差异性表达的 microRNA 在角膜和皮肤上皮干细胞分化过程中的变化提示其在维持上皮干细胞干性和引导不同上皮干细胞特定分化过程中的重要作用。
3. miR-145 调控皮肤及角膜上皮细胞的增殖能力，这在对上皮细胞的异常增殖和成瘤的控制中可能起着重要作用。

关键词：角膜；皮肤；microRNA

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Abstract

Objective To identify the differentially expressed microRNAs between limbal epithelial stem cells (LESCs) and skin epithelial stem cells (SESCs) and investigate the functions of one of theses microRNAs.

Methods

1. 6 samples of clone cultured limbal epithelial stem cell and skin epithelial stem cell (each 3 samples respectively) were collected and analyzed by microRNA array. To identify differentially expressed miRNAs with statistically significance, we performed a volcano plot filtering between the two groups from the experiment. The threshold we used to screen up or down regulated miRNAs is fold change>=1.5 and P value <=0.05.
2. Varify the miRNA arrays identifed differentially expressed miRNAs by realtime PCR, and select those miRNAs with the same expression pattern and prominentfold change.
3. Investigate the change of the selected miRNAs during differentiation of LESCs and SESCs by real-time qPCR.
4. Transfect one of the selected miRNAs---miR-145 into SESCs, observeing morphologic changes by phase contrast microscopeand and detecting proliferating ability by MTT.
5. Target genes of miR-145 related to cell proliferation were predited by TargetScan and miRanda. Expression changes of the predited gene and the downstream gene after miR-145 transfection were detected by realtime PCR and Westernblot.

Results

1. 27 differentially expressed microRNAs were identified by miRNA array; 17 miRNAs were up regulated in LESCs, which are miR-30a，miR-1297，miR-4301，miR-4262，miR-199a-3p，miR-190，miR-302f，miR-338-5p，miR-484，miR-21，miR-376b，miR-26a，miR-140-3p，miR-18b，miR-145，

miR-652，miR-324-5p; 10 miRNAs were up regulated in SESCs, which are miR-23a*，miR-943，miR-1236，miR-193b*，miR-382，miR-542-3p，miR-205*，miR-224*,miR-378，miRPlus-1382*.

2. 8 miRNAs (miR-4262，miR-199a-3p，miR-190，miR-302f，miR-21，miR-376b，miR-140-3p，miR-145) were seleted by realtime PCR with the same expression pattern and prominent fold change, all of which are up regulated in LESCs.
3. 6 of the 8 selected differentially expressed miRNAs were down regulated remarkably during both LESCs and SESCs differetiation, while 2 miRNAs, miR-302f and miR-4262 were up regultaed dramatically during LESCs differentiation but down regulated dramatically during SESCs differentiation.
4. miR-145 was selected to undergo function investigation and results showed proliferative inhibition by miR-145 over-expression in SESCs.
5. Futher mechanism studies indicated that the cell proliferative inhibition of miR-145 is through inhibition of p38 MAPK signaling pathway by down regulating miR-145 target gene p38, and along with down regulating of p38 MAPK downstream gene p63.

Conclusion

1. The differentially expressed miRNAs between LESCs and SESCs might play vital roles in keeping their stemness and respective properties.
2. The different changes of the selected differentaily expressed miRNAs during LESCs and SESCs differentiation indicated their functions in directing respctive differetiations of LESCs and SESCs.
3. Proliferative inhibitation of miR-145 in corneal and skin epithelial cell indicated its role in control of prolferation and tumorigenesis.

Keywords Limbal epithelial stem cells; Skin epithelial stem cells; microRNAs

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